BIO-RAD

# MONOLISA™ Anti-HBs EIA 25220
# MONOLISA™ Anti-HBs Calibrator Kit 25219

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**Enzyme Immunoassay (EIA) for the Qualitative and Quantitative Detection of Antibody to Hepatitis B Surface Antigen (anti-HBs) in Human Serum and EDTA or Citrated Plasma**

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**For In Vitro Diagnostic Use**

**MONOLISA™ Anti-HBs EIA • 192 Tests**
**MONOLISA™ Anti-HBs Calibrator Kit • 20 Tests**

## LEXICON

| | |
|---|---|
| WASH | Wash Solution Concentrate (30X) |
| TMB SOLUTION | Chromogen: TMB Solution |
| SUB BUF | Substrate Buffer |
| STOP | Stopping Solution |

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## 1 - NAME AND INTENDED USE

The Bio-Rad MONOLISA™ Anti-HBs EIA is a qualitative and quantitative enzyme immunoassay for the detection of antibody to hepatitis B surface antigen in human serum and EDTA or citrated plasma. The assay results may be used as an aid in the determination of susceptibility to hepatitis B virus (HBV) infection in individuals prior to or following HBV vaccination or where vaccination status is unknown. Assay results may be used with other HBV serological markers for the laboratory diagnosis of HBV disease associated with HBV infection. A reactive assay result will allow a differential diagnosis in individuals displaying signs and symptoms of hepatitis in whom etiology is unknown.

**WARNING:** This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients. The user is responsible for establishing their own assay performance characteristics in these populations

## 2 - SUMMARY AND EXPLANATION OF THE TEST

Hepatitis B Virus (HBV) is a major public health problem, with more than 400 million people chronically infected worldwide.[1] Chronic hepatitis B is a leading cause of cirrhosis and liver cancer. The virus is transmitted efficiently by a number of routes, including passage from mother to child and percutaneous or permucosal exposure to infectious blood or body fluids.[2] Sexual contact, intravenous drug use, blood transfusion, tissue transplantation, and hemodialysis procedures may transmit the disease.[3,4] Immunization with a licensed HBV vaccine is a highly effective strategy to prevent HBV transmission, with protection achieved in over 95% of all vaccinees.[5,6]

The genetic organization, transcription, and replication of the virus are well understood.[7,8] The whole virion, or Dane particle, contains an envelope, consisting of a lipid bilayer and glycoproteins (surface antigens), and a core (nucleocapsid) that encloses a circular DNA genome. The viral DNA encodes at least seven proteins from four open reading frames [surface (S), core (C), polymerase (P), and the X gene (X)]. The proteins that are important diagnostically are surface antigen (HBsAg), core antigen (HBcAg) and e antigen (HBeAg), a hidden epitope released by disruption of the nucleocapsid.

During the early stages of primary infection by hepatitis B virus, HBV DNA, as well as HBsAg and HBeAg, are readily detectable. As the host mounts an immune response, the first antibodies to appear are antibodies to the core antigen (anti-HBc), followed by anti-HBe and finally anti-HBs, which marks the immune stage.[9] In adults with normal immune function, most (94%-98%) recover completely from newly acquired HBV infection, clearing the virus from the blood and producing neutralizing antibodies during convalescence.[10,11] However, in some patients, HBsAg may persist for months or years and anti-HBs is undetectable, indicating a chronic disease state. The clinical course for an individual patient may be influenced by genetic differences in the host immune response, as well as the patient's age at the time of infection, sex, treatment with immunosuppressive agents, and the appearance of HBV mutants.

The presence of anti-HBs antibodies is an important factor in the diagnosis and prognosis of HBV infection, indicating previous exposure to HBV and acquired immunity. Anti-HBs is used in epidemiological surveillance studies, to assess past exposure to Hepatitis B in potential Hepatitis B vaccine recipients, to monitor the vaccination process, and to select plasma with high antibody concentrations for the manufacture of therapeutic immune globulin. The determination of anti-HBs levels has been standardized by means of the WHO Anti-HBs Reference Preparation, and a level greater than or equal to 10 milli-International Units per milliliter (10 mIU/mL) is considered protective against HBV infection. The verification of at least a minimum anti-HBs titer of 10 mIU/mL, i.e., an immunity threshold titer, is crucial for the appropriate management of vaccinated individuals who may subsequently be exposed to HBsAg-positive fluids and specimens.

## 3 - BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The MONOLISA™ Anti-HBs EIA is an enzyme immunoassay (direct antibody sandwich format) which utilizes polystyrene microwells coated with native HBsAg (human, subtypes *ad* and *ay*) as the solid phase and a conjugate containing horseradish peroxidase-labeled HBsAg (human, subtypes *ad* and *ay*). In the assay procedure, patient specimens and controls are incubated in the antigen-coated microwells. If antibodies to HBs are present in a specimen or control, they bind to the antigen. Excess sample is removed by a wash step. The conjugate is then added to the microwells and allowed to incubate. The conjugate binds to any antigen-antibody 31

complexes present in the microwells. Excess conjugate is removed by a wash step, and a chromogen/substrate solution is added to the microwells and allowed to incubate. If a sample contains anti-HBs, the bound enzyme (HRP) causes the colorless tetramethylbenzidine (TMB) in the chromogen solution to change to blue. The blue color turns yellow after the addition of a stopping solution. If a sample does not contain anti-HBs, the chromogen/substrate solution in the well remains colorless during the substrate incubation, and after addition of the stopping solution. The color intensity, measured spectrophotometrically, is proportional to the amount of anti-HBs present in the specimen. Absorbance value readings for patient specimens are compared to a cutoff value determined by the 10 mIU/mL calibrator, which is calibrated against the WHO reference standard.

## 4 - REAGENTS

**MONOLISA™ Anti-HBs EIA Product Description**

Product No. 25220 (192 test kit)

| Component | Contents | Preparation |
|---|---|---|
| R1 • Anti-HBs Microwell Strip Plates (2) | • Microwell strips in holder, coated with HBsAg (human *ad* and *ay* subtypes)<br>• Tabs are labeled "EE"<br>• ProClin® (trace) | Use as supplied. Return unused strips to the pouch. Do not remove desiccant. |
| R2 • Wash Solution Concentrate (30X)<br>1 bottle (120 mL) | • Sodium Chloride<br>• Tween 20 | Dilute 1:30 with deionized water. Clinical laboratory reagent water Type I or Type II is acceptable. |
| R3 • Specimen Diluent<br>1 bottle (10 mL) | • Fetal calf serum<br>• Buffer with protein stabilizers<br>• ProClin® 300, 0.1%<br>• Sample indicator dye | Use as supplied. |
| C0 • Anti-HBs Negative Control<br>1 vial (0.8 mL) | • Human serum; negative for HIV and HCV antibodies and HBsAg<br>• Gentamicin, 0.005%<br>• ProClin® 950, 0.16% | Use as supplied. |
| C1 • Anti-HBs Positive Control<br>1 vial (0.8 mL) | • Anti-HBs Immunoglobulin (Human) therapeutic grade, approximately 120 mIU/mL<br>• Human serum; negative for HIV and HCV antibodies and HBsAg<br>• Gentamicin, 0.005%<br>• ProClin® 950, 0.16%<br>• Red dye | Use as supplied. |
| C3 • 10 mIU/mL Calibrator<br>1 vial (1.8 mL) | • Buffer with protein stabilizers<br>• Fetal Bovine Serum<br>• Bovine Serum Albumin<br>• ProClin® 950, 0.16%<br>• Anti-HBs Immunoglobulin (Human), therapeutic grade, 10 mIU/mL<br>• Yellow dye | Use as supplied. |
| R4 • Anti-HBs Conjugate Concentrate (11X)<br>1 bottle (2.6 mL) | • HBsAg (human ad and ay subtypes) conjugated to HRP<br>• Buffer with protein stabilizers<br>• Gentamicin, 0.005%<br>• ProClin® 300, 0.5%<br>• Green dye | Dilute in Anti-HBs Conjugate Diluent as described. |
| R5 • Conjugate Diluent<br>1 bottle (26 mL) | • Buffer with protein stabilizers<br>• Calf Serum<br>• ProClin® 300, 0.1% | Ready to use as described under Working Conjugate Solution |
| R8 • Substrate Buffer<br>1 bottle (120 mL) | • Hydrogen Peroxide<br>• Citric Acid/Sodium Acetate buffer<br>• Dimethylsulfoxide (DMSO) | Use as supplied. |
| R9 • Chromogen (11X)<br>1 bottle (12 mL) | • Tetramethylbenzidine (TMB)* | Dilute with Substrate Buffer as described. |
| R10 • Stopping Solution<br>1 bottle (120 mL) | • 1N $H_2SO_4$ | Use as supplied. |
| Plate Sealers | • Clear plastic sealers | Use as supplied. |

*NOTE: Tetramethylbenzidine is a non-carcinogenic and non-mutagenic chromogen for peroxidase.[12,13]

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Store the kit at 2-8°C. Bring all reagents except Conjugate Concentrate to room temperature (18-30°C) before use. Return reagents to 2-8°C immediately after use. Store all unused strips/plates in pouch and reseal. Do not remove desiccant. Store strip plates at 2-8°C.

## 5 - WARNINGS

**For in vitro diagnostic use only.**

1. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivates, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[14] Biosafety Level 2[15] or other appropriate biosafety practices[16,17] should be used for materials that contain or are suspected of containing infectious agents. The following human blood derivatives are found in this kit:
   - 1.1 Human source material used in the preparation of the Negative Control (C0) and as a diluent for the Positive Control (C1) is nonreactive for detectable hepatitis B surface antigen (HBsAg), and antibodies to hepatitis B core antigen, hepatitis C virus (HCV), and human immunodeficiency viruses (HIV-1 and HIV-2).
   - 1.2 The human anti-HBs immunoglobulin used in the preparation of the Positive Control (C1) and Calibrator (C3) is a therapeutic grade material which has been inactivated.
   - 1.3 The human plasma derived viral antigen HBsAg subtypes ad and ay used in the preparation of the Microplate (R1) and Conjugate Concentrate (R4) are highly purified and heat treated.
2. The following is a list of potential chemical hazards contained in some kit components (See section 4: REAGENTS):
   - 2.1 ProClin® 300 (0.1% or 0.5%) or ProClin® 950 (0.16%) are biocidal preservatives that are irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
   - 2.2 0.005% Gentamicin Sulfate, a biocidal preservative, which is a known reproductive toxin, photosensitizer and sensitizer; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
   - 2.3 The 1.0 N Sulfuric Acid ($H_2SO_4$) Stopping Solution is irritating to skin and severely irritating or corrosive to eyes, depending on the amount and length of exposure; greater exposures can cause eye damage, including permanent impairment of vision. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Keep away from strong bases, reducing agents and metals; do not pour water into this component. Waste from this material is considered hazardous acidic waste. However, if permitted by local, regional, and national regulations, it can be neutralized to pH 6-9 for non-hazardous disposal if operators are trained and equipped to do so.
3. Biological spills: Human source material spills should be treated as potentially infectious. Spills not containing acid should be immediately decontaminated, including the spill area, materials and any contaminated surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of bleach, 70-80% Ethanol or Isopropanol, an iodophor [such as 0.5% Wescodyne™ Plus], or a phenolic, etc.) and wiped dry.[18-20]

   Spills containing acid should be appropriately absorbed (wiped up) or neutralized, wiped dry and then the area should be decontaminated with one of the chemical disinfectants; materials used to absorb the spill should be disposed of as biohazardous waste.

   NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.
4. Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory chemical or biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.

## 6 - PRECAUTIONS FOR USERS

1. This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, including lab coat, eye/face protection and disposable gloves (synthetic, non-latex gloves are recommended) and handle with the requisite Good Laboratory Practices. Wash hands thoroughly after performing the test.
2. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
3. Do not pipette by mouth.
4. The Bio-Rad MONOLISA™ Anti-HBs EIA is intended for the detection of **antibody to hepatitis B** surface **antigen** and does not detect HBsAg. The tabs at the end of the microwell strips are labeled with product code "EE". The Bio-Rad Genetic Systems™ HBsAg EIA 3.0 is intended for the detection of **hepatitis B surface antigen**, and does not detect antibody to HBsAg.
5. Do not use any kit components beyond their stated expiration date.
6. Any lot number of the following reagents may be used with this assay provided they have the correct catalog number and are not used beyond their labeled expiration date:
   Chromogen (R9) – Catalog # 26182
   Substrate Buffer (R8) – Catalog # 26181
   Wash Solution Concentrate (R2) – Catalog # 25261
   Stopping Solution (R10) – Catalog # 25260

   **Do not mix any other reagents from different lot numbers.**
7. Do not use the Chromogen (R6), the Chromogen Diluent (R4) and/or the Buffered Substrate (R7a) color development solutions found in the Genetic Systems™ rLAV EIA and Genetic Systems™ HIV-2 EIA test kits.
8. Exercise care when opening vials and removing aliquots to avoid microbial contamination of the reagents.
9. Use a clean, disposable container for the conjugate. Exposure of the conjugate to sodium azide will result in its inactivation.
10. Avoid exposing Chromogen or Working TMB Solution to strong light during storage or incubation. Do not allow the Working TMB Solution to come into contact with any oxidizing agents.
11. Avoid contact of the Stopping Solution with any oxidizing agent. Do not allow Stopping Solution to come into contact with metals.
12. Use clean, polypropylene containers to prepare and store the Working TMB Solution. If glassware must be used, pre-rinse thoroughly with 1N sulfuric or hydrochloric acid followed by at least three washes of deionized water. Be sure that no acid residue remains on the glassware.
13. For the manual pipetting of controls and specimens, use individual pipette tips to eliminate carryover of samples.
14. Handle the Negative and Positive Controls and the 10 mIU/mL Calibrator in the same manner as patient specimens.
15. Use only adequately calibrated equipment with this assay.
16. Use of dedicated equipment is recommended if equipment performance validations have not precluded the possibility of cross-contamination.
17. The MONOLISA™ Anti-HBs EIA Procedure and the Interpretation of Results must be followed when testing serum or plasma specimens for the presence of antibodies to HBsAg. The user of this kit is advised to read the package insert carefully prior to conducting the test. In particular, the test procedure must be carefully followed for sample and reagent pipetting, plate washing, and timing of the incubation steps. Inadequate adherence to package insert instructions may result in erroneous results.
18. Failure to add specimen or reagent as instructed in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of procedural error.
19. An absorbance value of less than 0.000 AU may indicate a procedural or instrument error that should be evaluated. That result is invalid and that specimen must be re-run. If repeated results are < 0.000, the performance of the instrumentation should be investigated.

20. Factors that can affect the validity of results include failure to add the specimen to the well, inadequate washing of microplate wells, failure to follow stated incubation times and temperatures, addition of wrong reagents to wells, the presence of metals, or splashing of bleach into wells.

## 7 - REAGENT PREPARATION AND STORAGE

### Working Conjugate Solution (R4 + R5)

Bring Conjugate Diluent (R5) to room temperature. Invert Diluent (colorless to pale straw) and Conjugate Concentrate (R4, green) to mix before using. Prepare a 1:11 dilution for each strip to be tested by adding 100 µL of Conjugate Concentrate to each 1 mL of Conjugate Diluent in a clean, polypropylene tube. Use the following table as a guide. Mix well but gently to avoid foaming. Working Conjugate Solution should be green. Note Concentrate lot number, date and time of preparation, and date and time of expiration of the Working Conjugate Solution. Working Conjugate Solution is stable for 8 hours at room temperature, and for 1 month if stored at 2-8°C. Alternatively, Working Conjugate Solution can be prepared by pipetting the entire contents of the Conjugate Concentrate vial into the Conjugate Diluent. Working Conjugate Solution should be protected from light, both at room temperature and at 2-8°C. Always mix working solution by inverting just prior to use.

Return unused Conjugate Concentrate to the refrigerator immediately after use. To avoid contamination of Conjugate, wear clean gloves and do not touch tips of pipettes.

Prepare only the amount of the reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Use the following table as a guide:

**Preparation of Working Conjugate Solution by Number of Strips Used**

| Number of Strips to be used | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12* | 24** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amount of Conjugate Concentrate (µL) | 100 | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 2400 |
| Amount of Conjugate Diluent (mL) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 24 |

* 1 Complete Plate ** 2 Complete Plates

### Working TMB Solution (R8 + R9)

Bring Chromogen (R9) and Substrate Buffer (R8) to room temperature. Invert the Chromogen and Substrate Buffer to mix before using. Prepare a 1:11 dilution for each strip to be tested by mixing 100 µL of Chromogen to each 1 mL of Substrate Buffer in a clean, polypropylene container. Note Chromogen lot number, date and time of preparation, and date and time of expiration (8 hours from preparation) on container. Mix TMB Working Solution gently prior to use. Working TMB Solution should be kept in the dark at room temperature and used within 8 hours of preparation.

Chromogen should be colorless. Any other color indicates that the reagent is compromised and should not be used. Prepare only the amount of the reagent to be used within 8 hours, ensuring that the volume of diluted reagent will be adequate for the entire run. Extra Chromogen is provided. Use the following table as a guide:

**Preparation of Working TMB Solution by Number of Strips Used**

| Number of Strips to be used | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12* | 24** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amount of Chromogen (µL) | 100 | 200 | 300 | 400 | 500 | 600 | 700 | 800 | 900 | 1000 | 1100 | 1200 | 2400 |
| Amount of Substrate Buffer (mL) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 24 |

* 1 Complete Plate ** 2 Complete Plates

### Wash Solution (R2)

Prepare Wash Solution (R2) by adding one part Wash Solution Concentrate (30X) to 29 parts of deionized or distilled water (e.g., 120 mL of Wash Solution Concentrate to 3480 mL of deionized water). Clinical laboratory reagent water Type I or Type II is acceptable. The diluted Wash Solution can be stored ambient for up to four weeks in a plastic container. Note the lot number, date prepared, and expiration date on the container. Discard if no suds are evident in the Wash Solution. Prepare a sufficient quantity of Wash Solution to complete a full run.

## 8 - SPECIMEN COLLECTION, PREPARATION, AND STORAGE

Serum or certain types of plasma may be used in the test. The following tube types and anticoagulants, including those in both glass and plastic tubes, have all been evaluated and found to be acceptable: SST, EDTA, and sodium citrate. Specimens that are collected into anticoagulant tubes should be filled as labeling indicates to avoid improper dilution. The volume of anticoagulant in Na citrate tubes causes a specimen dilutional effect. Individuals with borderline results obtained from specimens collected in Na citrate should be retested using serum specimens. Specimens with observable particulate matter should be clarified by centrifugation prior to testing.

Serum/plasma should remain at 22°C for no longer than eight hours. If assays are not completed within eight hours, serum/plasma should be refrigerated at 2 to 8°C. Specimens may be stored at 2-8°C for 48 hours. For long-term storage, the specimens should be frozen (at -20°C or lower). Specimens should not be used if they have incurred more than 5 freeze-thaw cycles. Mix specimens thoroughly after thawing.

*Note: If specimens are to be shipped, they should be packed in compliance with Federal Regulations covering the transportation of etiologic agents.* Specimens should be kept frozen (-20°C or lower) for shipment.

## 9 – MONOLISA™ Anti-HBs EIA PROCEDURE

### Materials Provided

See REAGENTS section on page 4.

### Optional Materials

MONOLISA™ Anti-HBs Calibrator Kit (Catalog # 25219)

### Materials Required But Not Provided

1. Precision pipettes to deliver volumes from 25 µL to 200 µL, 1 mL, 5 mL, and 10 mL (accurate within ± 10%). A multichannel pipettor capable of delivering 100 µL is optional.
2. Pipette tips.
3. Appropriately sized graduated cylinders.
4. Dry-heat incubator capable of maintaining 37 ± 2°C.
5. Bio-Rad microwell plate or strip washer, or equivalent. The washer must be capable of dispensing 375 µL per well, cycling 5 times, and soaking for 30-60 seconds between each wash.
6. Bio-Rad microwell plate or strip reader or equivalent. The spectrophotometer should have the following specifications at wavelengths 450 nm and 405 nm:
   Bandwidth: 10 nm HBW (Half Band Width) or equivalent
   Absorbance Range: 0 to 2 AU (Absorbance Units)
   Repeatability: ± (0.5% + 0.005) AU
   Linearity or Accuracy: 1% from 0 to 2.0 AU
   The instrument should contain a reference filter for reading at 615 nm to 630 nm. An instrument without a reference filter can be used; however, areas in the bottoms of the wells that are opaque, scratched or irregular may cause absorbance readings that are falsely elevated.
7. Household bleach (5% to 8% sodium hypochlorite) may be diluted to a minimum concentration of 10% bleach (or 0.5% sodium hypochlorite). Alternative disinfectants include: 70% ethanol or 0.5% Wescodyne™ Plus (West Chemical Products, Inc.).
8. Paper towels or absorbent pads for blotting.
9. Labeled null strips, for testing partial plates.
10. Clean, polypropylene containers of appropriate size for the preparation of TMB (do not use polystyrene) and Conjugate Working Solutions.
11. Deionized or distilled water. Clinical laboratory reagent water Type I or Type II is acceptable.
12. Gloves.
13. Laboratory timer.
14. EIA reagent reservoirs (optional).

**Preliminary Statements**

1. The expected run time for this procedure is approximately 3 hours from initiation of the first incubation step. Each run of this assay must proceed to completion without interruption after it has been started.
2. Controls to be included on each plate of this assay: Positive Control (run singly), Negative Control (run singly), and the 10 mIU/mL Cutoff Calibrator (run in triplicate). The cutoff for patient specimens is determined by the mean ($\bar{x}$ ) value of the 10 mIU/mL Calibrator replicates on each individual plate.
3. Specimens, Calibrators and Controls may be diluted in-well by one of two methods:
   i. Add 25 µL of Specimen Diluent to each well first, followed by 75 µL of specimen or control within 30 minutes, then mix gently to avoid foaming.
   ii. Add 75 µL of specimen or control to each well first, followed by 25 µL of Specimen Diluent within 30 minutes, then mix gently to avoid foaming.
   Note: To maintain consistency in results, use only one of the above methods per plate.
4. The procedure specifies the addition of 100 µL volumes of diluted specimen, Working Conjugate Solution, Working TMB Solution and Stopping Solution while performing the assay. No adverse effects were noted when volumes from 90 – 150 µL were tested at each of these steps.
5. Do not splash controls, specimens, or reagents between microwells of the plate.
6. Cover plates for each incubation step using plate sealers provided or other appropriate means to minimize evaporation.
7. Avoid exposure of the plates to light during the final incubation step (following the addition of the Working TMB Solution).
8. Adhere to the recommended time constraints for the use of the Working TMB Solution (8 hours, ambient), Working Conjugate Solution (8 hours at ambient temperature, or 1 month at 2-8°C), and Wash Solution (4 weeks, ambient).
9. Avoid the formation of air bubbles in each microwell.

## EIA Procedure

The MONOLISA™ Anti-HBs EIA performance is dependent upon incubation times and temperatures. Temperatures outside of the validated ranges may result in invalid assays. Incubation temperatures should be carefully monitored using calibrated thermometers, or equivalent.

1. Perform equipment maintenance and calibration, where necessary, as required by the manufacturer.
2. **Bring all of the reagents except Conjugate Concentrate to room temperature before beginning the assay procedure.**
3. Prepare Working Conjugate Solution, Working TMB Solution, and Working Wash Solution. Mix gently, by inversion.
4. Remove strips not needed for the assay and replace them with labeled Null Strips, as necessary.
5. If specimen identity is not maintained by an automatic procedure, identify the individual wells for each specimen or control on a data sheet.
6. **Dilute specimens, calibrators and controls 3:4 in the Specimen Diluent:**
   **Specimens, calibrators and controls may be prediluted 3:4** in the Specimen Diluent prior to addition to the well (for example, dilute 150 µL of specimen in 50 µL of Specimen Diluent, mix gently to avoid foaming, and then transfer **100 µL** to the well), or **diluted in-well.** (See previous Preliminary Statement No. 3 for details.)
   ***NOTE:*** *After adding the specimen, the diluent will change from purple to a blue color.*
   It is possible to verify the presence of samples in the wells by spectrophotometric reading at 615 nm to 630 nm (single wavelength). Refer to Section 10: Spectrophotometric Verification of Sample and Reagent Pipetting (optional).
   One well of Positive Control, one well of Negative Control and three wells of the 10 mIU/mL Cutoff Calibrator must be assayed on each plate or partial plate of specimens when performing the qualitative procedure.
7. Cover the microwell plate with a plate sealer or use other means to minimize evaporation. **Incubate the plate for 60 ± 5 minutes at 37 ± 2 °C.**

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8. At the end of the incubation period, carefully remove the plate sealer and aspirate the fluid from each well into a biohazard container. **Wash the microwell plate or strip a minimum of five times** with the Wash Solution (at least 375 µL/well/wash), or as otherwise validated. **Soak each well for 30 to 60 seconds between each wash cycle.** Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on clean, absorbent paper towels. ***NOTE:*** *Grasp the plate holder firmly at the center of the long sides before inverting to blot.*
9. **Add 100 µL of the Working Conjugate Solution to each well containing a specimen, calibrator or control.** Avoid bumping plates containing working conjugate solution to prevent contamination of the plate sealer and/or top edges of the wells.
   ***NOTE:*** *The conjugate is colored green.*
   It is possible to verify the presence of conjugate in the wells by spectrophotometric reading at 615 nm to 630 nm (single wavelength). See Section 12: Spectrophotometric Verification of Sample and Reagent Pipetting.
10. Cover the microwell plate with a plate sealer or use other means to minimize evaporation. **Incubate the plate for 60 ± 5 minutes at 37 ± 2 °C.**
11. At the end of the incubation period, carefully remove the plate cover and aspirate the fluid in each well into a biohazard container. **Wash the microwell plate or strip a minimum of five times** with the Wash Solution (at least 375 µL/well/wash), or as otherwise validated. **Soak each well for 30 to 60 seconds between each wash cycle.** Aspirate the Wash Solution after each wash. After the last wash, if excess liquid remains, blot the inverted plate on a clean, absorbent paper towel.
    ***NOTE:*** *Grasp the plate holder firmly at the center of the long sides before inverting to blot.*
12. **Add 100 µL of the Working TMB Solution to each well containing a specimen, calibrator, or control. Incubate plates in the dark for 30 ± 5 minutes at room temperature (18 to 30°C).** (For example, cover the plates with black plastic or place in a drawer).
13. **Add 100 µL of Stopping Solution to each well** to terminate the reaction. Use the same sequence and rate of distribution as for the substrate solution addition. **Tap the plate gently, or use other means to assure complete mixing. Complete mixing is required for acceptable results.**
14. Carefully wipe the plate bottom and ensure that all strips have been pressed firmly into place before reading. **Read absorbance within 30 minutes after adding the Stopping Solution,** using the **450 nm filter with 615 nm to 630 nm** as the reference. (Blank on air.)

**Decontamination**

Dispose of all specimens and materials used to perform the test as though they contain an infectious agent. Disposal should comply with all applicable waste disposal requirements.

## 10 - SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETTING (OPTIONAL)

VERIFICATION OF SAMPLE PIPETTING

After the sample addition to the Specimen Diluent, the purple diluent turns blue.
The presence of sample in the well can be verified by spectrophotometric reading at 615 nm to 630 nm (single wavelength).

- The O.D. values of the wells containing sample or control diluted in Specimen Diluent (R3) must be greater than or equal to 0.150. A value lower than this indicates poor dispensing of the sample or control.

VERIFICATION OF THE CONJUGATE DISPENSE

The Conjugate (R4) is green in color.
The presence of Conjugate (R4) in the well can be verified by spectrophotometric reading at 615 nm to 630 nm (single wavelength):

- The O.D. value of each well must be greater than or equal to 0.100. A value lower than this indicates poor dispensing of the Working Conjugate Solution.

## 11 – QUALITY CONTROL – VALIDATION OF RESULTS

Each plate should contain a Positive Control, a Negative Control, and three Cutoff Calibrators. The Positive and Negative Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff. In addition, the quality control supplied in the MONOLISA Anti-HBs EIA is in a serum matrix and may not adequately control the assay for plasma specimens. The user should include alternate control material for plasma matrices.

The test is invalid and must be repeated if the absorbance readings of the controls and the calibrator do not meet specifications. If the test is invalid, patient results cannot be reported. Quality control testing must be performed in conformance with local, state, and/or federal regulations, or accreditation requirements, and your laboratory's standard Quality Control procedures. It is recommended that the user refer to NCCLS C24-A and 42 CFR 493.1256 for guidance on appropriate QC practices.

The 10 mIU/mL Cutoff Calibrators must meet both the absorbance and precision criteria listed below.

The individual absorbance value of each 10 mIU/mL Cutoff Calibrator must be greater than or equal to 0.050 and less than or equal to 0.150.

Individual Cutoff Calibrator absorbance values must be within the range 0.65 X $CAL_{10}\bar{x}$ to 1.35 X $CAL_{10}\bar{x}$ .

If the Cutoff Calibrators do not meet these acceptance criteria, the run is invalid and must be repeated.

**Cutoff Value Example:**

10 mIU/mL Cutoff Calibrator

| Sample Number | Absorbance | Total Absorbance | = 0.332 | = 0.111 ($CAL_{10}\bar{x}$ ) |
|---|---|---|---|---|
| 1 | 0.113 | 3 | 3 | |
| 2 | 0.109 | | | |
| 3 | 0.110 | | | |
| | 0.332 | | | |

All three 10 mIU/mL Cutoff Calibrator values above are within the range of 0.65 to 1.35 times the $CAL_{10}\bar{x}$ as shown by the calculation below:

0.65 X $CAL_{10}\bar{x}$ = 0.65 X 0.111 = 0.072

1.35 X $CAL_{10}\bar{x}$ = 1.35 X 0.111 = 0.150

Therefore, the acceptance range is 0.072 to 0.150.

**The mean absorbance of the 10 mIU/mL Cutoff Calibrators ($CAL_{10}\bar{x}$ ) is the Cutoff Value for the assay.**

**Assay Validation**

A run is valid if the following criteria are met:

- The absorbance value of the Positive Control must be greater than or equal to 0.600 AU (PC ≥ 0.600).
- The individual absorbance value of each 10 mIU/mL Cutoff Calibrator ($CAL_{10}i$) must be greater than or equal to 0.050 and less than or equal to 0.150 AU (0.050 ≤ $CAL_{10}i$ ≤ 0.150). All Cutoff Calibrator absorbance values must also be within the range 0.65 X $CAL_{10}\bar{x}$ to 1.35 X $CAL_{10}\bar{x}$ (0.65 X $CAL_{10}\bar{x}$ ≤ $CAL_{10}i$ ≤ 1.35 X $CAL_{10}\bar{x}$ ). If the Cutoff Calibrators fail to meet these acceptance criteria, the run is invalid and must be repeated.
- The absorbance value of the Negative Control must be greater than 0.000 AU and less than or equal to 0.100 AU (0.000 < NC ≤ 0.100).
- The absorbance value of the Negative Control must be less than the mean of the absorbance values of the 10 mIU/mL Cutoff Calibrator, that is, less than the Cutoff Value (NC < $CAL_{10}\bar{x}$ ).

If any one of the above criteria is not met, the assay is invalid and must be repeated.

## 12 - INTERPRETATION OF RESULTS (Qualitative Procedure)

**Borderline:** Specimens with antibody levels of 9-11 mIU/mL should be interpreted as borderline, as the specific immune status for those patients can't be determined without other clinical information or subsequent testing. The borderline interpretation zone is calculated based on the mean of the 10 mIU/mL Cutoff Calibrator. Specimens that are within ±10% of the Cutoff Calibrator mean O.D. (90% ≤ $CAL_{10}\bar{x}$ O.D. ≤110%) are borderline.

For specimens that are borderline the subject can be re-collected in 2-3 weeks for additional testing. In conjunction with these results, the immune status of subjects should be evaluated based on their clinical status, related risk factors, and other diagnostic test results.

**Reactive:** Specimens with absorbance values greater than the borderline zone (>11 mIU/mL) are considered reactive, and the patient is considered to be immune to infection with HBV. It has not been determined what the clinical significance is for values greater than 11 mIU/mL, other than the individual is considered to be immune to HBV infection.

**Nonreactive:** Specimens with absorbance values less than the borderline zone are considered nonreactive, and the patient is considered to be not immune to infection with HBV. The absorbance value of a specimen must be compared to the borderline zone determined for the microwell plate on which it is assayed.

**Example:**

| | | | |
|---|---|---|---|
| Positive Control O.D. Value | **1.154** | | Valid |
| 10 mIU/mL Cutoff Calibrator Individual O.D. values | 0.113<br>0.109<br>0.110 | $CAL_{10}\bar{x}$ : | 0.111<br>Valid |
| Negative Control O.D. value | 0.036 | | Valid |
| Borderline O.D. zone | 0.100 – **0.122** | *Cutoff Calibrator ($CAL_{10}\bar{x}$ ) ±10%* | |
| Specimen O.D. values | **2.453**<br>0.040<br>0.018<br>0.115<br>**0.215** | *Interpretation:* | Reactive<br>Nonreactive<br>Nonreactive<br>Borderline<br>Reactive |

Specimens with absorbance values that are less than 0.000 must be repeated. Those with values greater than the upper linearity limits of the reader should be reported as reactive.

**The volume of anticoagulant in Na citrate tubes causes a specimen dilutional effect. Individuals with borderline results obtained from specimens collected in Na citrate should be retested using serum specimens.**

## 13 - LIMITATIONS

1. For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
2. A non-reactive test result does not exclude the possibility of exposure to hepatitis B virus.
3. Results obtained with the MONOLISA™ Anti-HBs EIA assay may not be used interchangeably with values obtained with different manufacturers' Anti-HBs assay methods.
4. Results from immunosuppressed patients should be interpreted with caution.
5. This assay does not differentiate between a vaccine-induced immune response and an immune response induced by infection with HBV. To determine if the anti-HBs response is due to vaccine or HBV infection, a total anti-HBc assay may be performed.
6. Performance characteristics have not been established for therapeutic monitoring.
7. A reactive anti-HBs result does not exclude co-infection by another hepatitis virus.
8. Individuals that have received blood component therapy (e.g., whole blood, plasma, immune globulin administration) during the previous 3 to 6 months may have a false reactive anti-HBs result due to passive transfer of anti-HBs.
9. The performance of the MONOLISA™ Anti-HBs EIA has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic, or pleural fluids.

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## 14 – PERFORMANCE CHARACTERISTICS

A multi-center clinical trial was conducted to evaluate the performance of the MONOLISA™ Anti-HBs Enzyme Immunoassay (EIA) and the MONOLISA™ Anti-HBs Calibrator Kit in human serum and plasma. A total of 1452 prospective subjects at high risk for viral hepatitis and/or showing signs/symptoms of HBV were included in the study. Of these 1452, 1373 were asymptomatic from a high-risk population and 79 reported signs or symptoms of HBV.

### Expected Values

The expected values that can be seen with the MONOLISA™ Anti-HBs EIA, by gender and age range, were determined during the evaluation of 1373 prospective asymptomatic subjects. All subjects (100%) were at high risk for viral hepatitis including intravenous drug users (N = 476), homosexual males (N = 144), sex workers (N = 171), prison history (N = 340), high risk sex partners (167), high risk occupation/health care workers (N = 85), hemodialysis (N = 58), hemophiliacs (3), and other (N = 470). Many had more than 1 high risk behavior or risk factor. One hundred seventy six (12.8%) of these high risk subjects also reported having received a full course of injections of an HBV vaccine. Subjects in the asymptomatic prospective population were from the following geographic locations: 459 from Los Angeles, CA, (33.4%), 57 from Santa Ana, CA (4.1%), 72 from Miami, FL (5.2%), 345 from Cocoa, FL (25.1%), 273 from San Francisco, CA (19.9%), and 167 from Seattle, WA (12.2%). The group was Caucasian (36.5%), Black or African American (41.1%), Hispanic or Latino (13.3%), Asian (4.2%), Native Hawaiian or other Pacific Islander (0.7%), and American Indian or Alaska Native (2.2%), with the remaining 2.0% represented by multiple ethnic groups or was unknown. The subjects were male (70.1%) and female (29.9%) and ranged in age from 18 to 81 years.

The MONOLISA™ Anti-HBs EIA results for the asymptomatic prospective population, by gender and age range, are presented in Table 1.

**Table 1**
**Expected Values by Gender and Age - MONOLISA™ Anti-HBs EIA**

| Age Range | Gender | MONOLISA™ Anti-HBs EIA Result | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | Reactive | | Borderline | | Non-reactive | | |
| | | N | % | N | % | N | % | N |
| 10-19 | F | 6 | 100.0% | 0 | 0.0% | 0 | NA | 6 |
| | M | 7 | 70.0% | 0 | 0.0% | 3 | 30.0% | 10 |
| 20-29 | F | 44 | 42.3% | 1 | 1.0% | 59 | 56.7% | 104 |
| | M | 48 | 39.0% | 0 | 0.0% | 75 | 61.0% | 123 |
| 30-39 | F | 42 | 37.5% | 4 | 3.6% | 66 | 58.9% | 112 |
| | M | 75 | 35.0% | 1 | 0.5% | 138 | 64.5% | 214 |
| 40-49 | F | 40 | 37.4% | 3 | 2.8% | 64 | 59.8% | 107 |
| | M | 162 | 45.8% | 7 | 2.0% | 185 | 52.3% | 354 |
| 50-59 | F | 33 | 51.6% | 4 | 6.3% | 27 | 42.2% | 64 |
| | M | 108 | 51.2% | 8 | 3.8% | 95 | 45.0% | 211 |
| 60-69 | F | 5 | 41.7% | 0 | 0.0% | 7 | 58.3% | 12 |
| | M | 23 | 57.5% | 0 | 0.0% | 17 | 42.5% | 40 |
| 70-79 | F | 1 | 50.0% | 0 | 0.0% | 1 | 50.0% | 2 |
| | M | 3 | 60.0% | 0 | 0.0% | 2 | 40.0% | 5 |
| 80-89 | F | 0 | NA | 0 | NA | 0 | NA | 0 |
| | M | 0 | NA | 0 | 0.0% | 1 | 100.0% | 1 |
| Unknown | F | 2 | 66.7% | 0 | 0.0% | 1 | 33.3% | 3 |
| | M | 1 | 20.0% | 1 | 20.0% | 3 | 60.0% | 5 |
| **Totals** | | **600** | **43.7%** | **29** | **2.1%** | **744** | **54.2%** | **1373** |

### Reference Markers

The HBV disease classification for each subject in the total prospective population (N = 1452) was determined by a serological assessment using a hepatitis marker profile consisting of FDA-approved commercially available reference EIAs. The six HBV reference marker assays included HBsAg, hepatitis B virus e antigen (HBeAg), total

antibody to hepatitis B virus core antigen (Anti-HBc, Total) IgM antibody to hepatitis B virus core antigen (Anti-HBc IgM), total antibody to HBe Ag (Anti-HBe), and total antibody to hepatitis B virus surface antigen (anti-HBs, qualitative or quantitative). All reference EIAs were tested according to the manufacturer's package insert instructions. Agreement of the MONOLISA™ Anti-HBs EIA was assessed relative to the reference anti-HBs result and to the reference HBV classification.

In the MONOLISA™ Anti-HBs EIA clinical study, across three clinical sites, there were 38 unique reference HBV marker patterns observed. Table 2 summarizes the HBV test patterns and their associated classifications. No other laboratory or clinical information was used in the HBV disease classification process.

**Table 2**
**Characterization of Prospective Specimens**

| FDA Characterization based on single point specimen | HBsAg | HBeAg | Anti-HBc IgM | Total HBc | Anti-HBe | Anti-HBs |
|---|---|---|---|---|---|---|
| Acute infection | + | + | + | + | - | - |
| Acute infection | + | + | - | - | - | - |
| Acute infection | + | - | + | - | - | + |
| Acute infection | + | - | - | - | - | - |
| Acute infection | + | - | + | - | - | - |
| Acute infection | + | + | I | + | - | - |
| Chronic infection | + | + | - | + | - | - |
| Chronic infection | + | + | - | + | - | + |
| Chronic infection | + | + | - | + | + | - |
| Chronic infection | + | + | + | + | - | + |
| Chronic infection | + | - | - | + | - | - |
| Chronic infection | + | - | - | + | - | + |
| Chronic infection | + | - | - | + | + | - |
| Chronic infection | + | - | - | + | + | + |
| Chronic infection | + | - | - | + | I | - |
| Chronic infection | + | + | - | + | + | + |
| Early recovery | - | - | - | + | - | - |
| Early recovery | - | - | - | + | + | - |
| Early recovery | - | - | I | + | + | + |
| Early recovery | - | - | - | + | I | - |
| Early recovery | - | - | + | + | - | + |
| Early recovery | - | - | + | + | + | - |
| Early recovery | - | - | + | + | + | + |
| HBV vaccine response | - | - | - | - | - | + |
| HBV vaccine response (?) | - | - | - | - | - | I |
| Not previously infected with HBV | - | - | - | - | - | - |
| Recovered | - | - | - | + | - | I |
| Recovered | - | - | - | + | I | I |
| Recovered or Immune due to natural infection | - | - | - | + | - | + |
| Recovery | - | - | - | - | + | + |
| Recovery | - | - | - | + | + | + |
| Recovery | - | - | - | + | + | I |
| Recovery | - | - | - | + | I | + |
| Uninterpretable | + | - | - | - | + | - |
| Uninterpretable | - | + | - | - | - | + |
| Uninterpretable | - | + | - | + | - | - |
| Uninterpretable | - | + | - | - | - | - |
| Uninterpretable | - | - | - | - | + | - |

(-) = Negative / Nonreactive, (+) = Positive / Reactive, (I) = Indeterminate

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### Comparison of Results

A comparison of the MONOLISA™ Anti-HBs EIA results with the reference anti-HBs assay for each specimen classification is shown in Table 3. In clinical studies, specimens that had indeterminate results on the reference test were retested in duplicate per the manufacturer's instructions for use. Any specimens with 2/3 or 3/3 results within the indeterminate range were classified as indeterminate on the reference test.

**Table 3**
**FDA HBV Classification of High Risk Prospective Specimens**
**MONOLISA™ Anti-HBs EIA versus Reference Anti-HBs EIA**

| Reference HBV Classification | Reference Anti-HBs Result | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | | | Indeterminate | | | Negative | | | |
| | MONOLISA Anti-HBs | | | MONOLISA Anti-HBs | | | MONOLISA Anti-HBs | | | |
| | R | BRD[2] | NR | R | BRD[2] | NR | R | BRD[2] | NR | |
| Acute infection | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 13 | **14** |
| Chronic infection | 4 | 1 | 1 | 0 | 0 | 0 | 4[1] | 0 | 71 | **81** |
| Early Recovery | 3 | 1 | 0 | 0 | 0 | 0 | 10 | 5 | 88 | **107** |
| Recovery | 160 | 3 | 4 | 3 | 4 | 2 | 0 | 0 | 0 | **176** |
| Recovered | 0 | 0 | 0 | 5 | 6 | 1 | 0 | 0 | 0 | **12** |
| Recovered or Immune due to natural infection | 91 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | **96** |
| HBV vaccine response | 307 | 1 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | **316** |
| HBV vaccine response (?) | 0 | 0 | 0 | 16 | 8 | 7 | 0 | 0 | 1 | **32** |
| Not previously infected with HBV | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 598[1] | **609** |
| Uninterpretable | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | **9** |
| **Total** | **567** | **7** | **17** | **24** | **18** | **10** | **25** | **5** | **779** | **1452** |

[1] Includes specimens that were NRR (not repeatedly reactive)
[2] BRD = Borderline (± 10% of cutoff value)

Overall 567 samples were positive on both assays, 18 samples were indeterminate/borderline on both assays, and 779 samples were negative on both assays.

### Percent Agreement

The percent agreement between the MONOLISA™ Anti-HBs EIA and the reference anti-HBs assays was evaluated for each specimen classification, including the upper and lower 95% Wilson confidence bounds. A summary of this analysis for the prospective population is presented for each HBV classification in Table 4.

**Table 4**
**Percent Agreement**
**MONOLISA™ Anti-HBs EIA versus Reference Anti-HBs EIA**

| HBV Classification | N =[1] | Positive Percent Agreement[2] | 95% Confidence Interval | Negative Percent Agreement[3] | 95% Confidence Interval |
|---|---|---|---|---|---|
| Acute Infection | **14** | 100.0% (1/1) | 20.7%, 100.0% | 100.0% (13/13) | 77.2%, 100.0% |
| Chronic Infection | **81** | 66.7% (4/6) | 30.0%, 90.3% | 94.7% (71/75) | 87.1%, 97.9% |
| Early Recovery | **107** | 75.0% (3/4) | 30.1%, 95.4% | 85.4% (88/103) | 77.4%, 91.0% |
| Recovery | **176** | 94.7% (160/169) | 90.2%, 97.2% | 0.0% (0/3) | NA |
| Recovered | **12** | 0.0% (0/1) | NA | 0.0% (0/5) | NA |
| Past Infection | **96** | 94.8% (91/96) | 88.4%, 97.8% | NA (0/0) | NA |
| HBV Vaccine response | **316** | 97.2% (307/316) | 94.7%, 98.5% | NA (0/0) | NA |
| HBV vaccine response (?) | **32** | 0.0% (0/7) | NA | 5.9% (1/17) | 1.0%, 27.0% |
| Not previously infected | **609** | NA (0/0) | NA | 98.2% (598/609) | 96.8%, 99.0% |
| Uninterpretable | **9** | 100.0% (1/1) | 20.7%, 100.0% | 100.0% (8/8) | 67.6%, 100.0% |
| Total | **1452** | **94.3% (567/601)** | **92.2%, 95.9%** | **93.5% (779/833)** | **91.6%, 95.0%** |

[1] N=Total number of samples; refer to Table 3 for correlation of borderline samples. The eighteen specimens that were indeterminate by both assays were not included in the percent agreement calculations. Positive or negative results from the MONOLISA™ Anti-HBs EIA were considered as non-agreements in the calculation of percent positive agreement and percent negative agreement when the corresponding reference assay result was indeterminate/borderline.
[2] Compares number of samples positive on both assays to sum of all positive samples on the reference assay + samples indeterminate on the reference assay and negative on MONOLISA™ Anti-HBs EIA.
[3] Compares number of samples negative on both assays to sum of all negative samples on the reference assay + samples indeterminate on the reference assay and positive on MONOLISA™ Anti-HBs EIA.

The positive percent agreement with the reference method is 94.3% (567/601) with a 95% confidence interval of 92.2 – 95.9%. The negative percent agreement with the reference method is 93.5% (779/833) with a 95% confidence interval of 91.6 – 95.0%.

**Seroconversion Panels**

The comparative sensitivity of the MONOLISA™ Anti-HBs EIA was determined by testing 4 commercially available Anti-HBV seroconversion panels and comparing the results to those in the associated certificates of analysis. Comparative results for only panel members near the point of seroconversion are presented in Table 5.

**Table 5**
**HBV Seroconversion Panel Results**

| Panel ID | Day since 1st bleed | Total # Members | MONOLISA™ Anti-HBs | | Reference Anti-HBs EIA[1] | |
|---|---|---|---|---|---|---|
| | | | S/CO | Result | S/CO | Result |
| RP016-08 | 60 | 20 | 0.26 | NR | 0.50 | NR |
| RP016-09 | 74 | | **1.02** | **BRD** | **1.47** | **R** |
| RP016-10 | 79 | | **1.95** | **R** | **2.43** | **R** |
| RP016-11 | 81 | | **3.72** | **R** | **2.43** | **R** |
| 6506-06 | 69 | 14 | 0.74 | NR | 0.8 | NR |
| 6506-07 | 83 | | **1.75** | **R** | **1.1** | **R** |
| 6506-08 | 97 | | **3.00** | **R** | **2.0** | **R** |
| 6514-09 | 112 | 17 | 0.57 | NR | 0.7 | NR |
| 6514-10 | 126 | | **1.20** | **R** | **1.3** | **R** |
| 6514-11 | 140 | | **1.91** | **R** | **1.4** | **R** |
| 6536-06 | 70 | 12 | 0.85 | NR | 0.7 | NR |
| 6536-07 | 84 | | **1.15** | **R** | 0.9 | NR |
| 6536-08 | 98 | | **1.42** | **R** | **1.3** | **R** |

[1] From Certificates of Analysis.
[2] BRD = Borderline

In 2 of the 4 seroconversion panels, the MONOLISA™ Anti-HBs EIA detected reactive levels of hepatitis B surface antibody at the same bleed as the reference anti-HBs EIA. In 1 of the 4 seroconversion panels the MONOLISA™ Anti-HBs EIA detected reactive levels of hepatitis B surface antibody 1 bleed before the reference anti-HBs EIA. One panel was borderline (S/CO = 1.02) on the MONOLISA™ Anti-HBs EIA at the first reactive bleed on the reference test.

**Clinical Performance with Individuals Who Received a Full Course of Hepatitis B Vaccine**
Retrospective studies were conducted to evaluate a total of 197 serum specimens from 197 subjects who had received a full course of 3 HBV vaccinations (SmithKline-Beecham Biologicals Engerix-B® HBV vaccine or Merck & Co., Inc. Recombivax HB® vaccine). Testing was compared to a reference anti-HBs EIA. The MONOLISA™ Anti-HBs EIA demonstrated immunity in 141/197 specimens or 71.6%, (95% confidence interval of 64.9% to 77.4%). The reference method demonstrated immunity in 134/197 specimens or 68.0%, (95% confidence interval of 61.2% - 74.1%).

**Table 6**
**Post-HBV Vaccination Results**

| | Reference Anti-HBs Result | | | |
|---|---|---|---|---|
| **MONOLISA™ Anti-HBs Result** | **Immune** | **Indeterminate** | **Not-Immune** | **Totals** |
| Immune | 134 | 3 | 4 | **141** |
| Borderline | 0 | 2* | 1 | **3** |
| Not-Immune | 0 | 1 | 52 | **53** |
| **Totals** | **134** | **6** | **57** | **197** |

* Two specimens that were indeterminate by both assays were not included in percent agreement calculations.

The positive percent agreement with the reference method is 99.3% (134/135) with a 95% confidence interval of 95.9 – 99.9%. The negative percent agreement with the reference method is 86.7% (52/60) with a 95% confidence interval of 75.8 – 93.1%.

**Clinical Performance with Matched Pre- and Post-HBV Vaccination Specimens**
In another study, matched sets of pre- and post-vaccination specimens from thirty-eight individuals who had received recombinant HBV vaccine (either SmithKline-Beecham Biologicals Engerix-B® HBV vaccine or Merck & Co., Inc. Recombivax HB® vaccine) were tested with the MONOLISA™ Anti-HBs EIA. The matched sets from each subject included four specimens. One specimen was a pre- vaccination specimen collected before receiving the first vaccination dose of HBV vaccine. The second and third specimens were collected right before the second vaccination dose and third vaccination dose respectively. A post vaccination specimen was collected a minimum of 2 weeks after receiving the full course of 3 injections.

**Pre- Vaccination Samples**
In pre-vaccination samples, one sample was reactive (immune) on the MONOLISA™ Anti-HBs EIA but nonreactive (not immune) on the reference assay. The negative percent agreement with the reference method is 97.4% (37/38) with a 95% confidence interval of 86.5 - 99.5%. Results are presented in Table 7 below.

**Table 7**
**Pre-Vaccination Specimen Results**
**MONOLISA™ Anti-HBs EIA versus Reference EIA**

| | Reference Anti-HBs Result | | |
|---|---|---|---|
| **MONOLISA™ Anti-HBs Result** | **I** | **NI** | **Total** |
| Immune | 0 | 1 | 1 |
| Borderline | 0 | 0 | 0 |
| Not Immune | 0 | 37 | 37 |
| **Total** | **0** | **38** | **38** |

I = Immune, NI = Not Immune

**Pre- Second Vaccination Samples**
In samples drawn just prior to the second vaccination in the series, the MONOLISA™ Anti-HBs EIA demonstrated immunity in 4/38 (10.5%) of the samples. The reference method demonstrated immunity in 1/38 (2.6%) of the samples.

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The positive percent agreement with the reference method is 100% (1/1) with a 95% confidence interval of 20.7 – 100%. The negative percent agreement with the reference method is 89.2% (33/37) with a 95% confidence interval of 75.3 – 95.7%. One sample was borderline on the MONOLISA™ Anti-HBs EIA assay. Results are presented in Table 8 below.

**Table 8**
**Pre-Second Vaccination Specimen Results**
**MONOLISA™ Anti-HBs EIA versus Reference EIA**

| MONOLISA™ Anti-HBs Result | Reference Anti-HBs Result | | |
|---|---|---|---|
| | I | NI | Total |
| Immune | 1 | 3 | 4 |
| Borderline | 0 | 1 | 1 |
| Not Immune | 0 | 33 | 33 |
| **Total** | **1** | **37** | **38** |

I = Immune, NI = Not Immune

**Pre- Third Vaccination Samples**

The positive percent agreement with the reference method is 100% (15/15) with a 95% confidence interval of 79.6 - 100%. The negative percent agreement with the reference method is 82.6% (19/23) with a 95% confidence interval of 62.9 – 93.0%. One sample was borderline on the MONOLISA™ Anti-HBs EIA assay. Results are presented in Table 9 below.

**Table 9**
**Pre-Third Vaccination Specimen Results**
**MONOLISA™ Anti-HBs EIA versus Reference EIA**

| MONOLISA™ Anti-HBs Result | Reference Anti-HBs Result | | |
|---|---|---|---|
| | I | NI | Total |
| Immune | 15 | 3 | 18 |
| Borderline | 0 | 1 | 1 |
| Not Immune | 0 | 19 | 19 |
| **Total** | **15** | **23** | **38** |

I = Immune, NI = Not Immune

**Post Vaccination Samples**

In samples drawn after the complete vaccination series (post vaccination), the positive percent agreement with the reference method is 97.0% (32/33) with a 95% confidence interval of 84.7 – 99.5%. The negative percent agreement with the reference method is 60% (3/5) with a 95% confidence interval of 23.1 – 88.2%. One sample that was immune and two that were not immune with the reference assay were borderline with the MONOLISA™ Anti-HBs EIA. Results are presented in Table 10 below.

**Table 10**
**Post-Vaccination Results**
**MONOLISA™ Anti-HBs EIA versus Reference EIA**

| MONOLISA™ Anti-HBs Result | Reference Anti-HBs EIA Result | | |
|---|---|---|---|
| | I | NI | Total |
| Immune | 32 | 0 | 32 |
| Borderline | 1 | 2 | 3 |
| Not Immune | 0 | 3 | 3 |
| **Total** | **33** | **5** | **38** |

I = Immune, NI = Not Immune

**Potentially Cross-reactive Medical Conditions**

The specificity of the MONOLISA™ Anti-HBs EIA assay was evaluated during the analysis of 393 serum specimens from individuals with unrelated medical conditions, representing 20 potentially cross-reacting

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conditions. All of the specimens were anti-HBs negative on another commercially available Anti-HBs assay. The results of each specimen tested on the MONOLISA™ Anti-HBs EIA are summarized in Table 11.

**Table 11**
**Potentially Cross-Reactive Medical Conditions**

| Clinical Condition | Nonreactive | Reactive | Borderline | Total |
|---|---|---|---|---|
| Autoimmune Diseases[1] | 20 | 0 | 0 | 20 |
| Cytomegalovirus (CMV) | 19 | 1[2] | 0 | 20 |
| Epstein Barr Virus (EBV) | 20 | 0 | 0 | 20 |
| Elevated liver enzymes | 3 | 0 | 0 | 3 |
| H. pylori positive | 10 | 0 | 0 | 10 |
| Hepatic cancer | 4 | 0 | 0 | 4 |
| Hepatitis A Infection (HAV) | 19 | 1[2] | 0 | 20 |
| Hepatitis C Infection (HCV) | 18 | 2[2] | 0 | 20 |
| Hepatitis D Infection (HDV) | 6 | 0 | 0 | 6 |
| Herpes Simplex Virus (HSV) | 20 | 0 | 0 | 20 |
| HIV-1 | 19 | 1[2] | 0 | 20 |
| HIV-2 | 15 | 4[3] | 1[4] | 20 |
| HTLV-I/II | 19 | 1[2] | 0 | 20 |
| Influenza Vaccine Recipients | 18 | 2[2] | 0 | 20 |
| Parvovirus B19 | 20 | 0 | 0 | 20 |
| Pregnant (bHCG positive) | 50 | 0 | 0 | 50 |
| Rheumatoid Factor (RF) | 20 | 0 | 0 | 20 |
| Rubella | 19 | 1[2] | 0 | 20 |
| SLE / ANA Positive | 19 | 1[2] | 0 | 20 |
| Syphilis | 20 | 0 | 0 | 20 |
| Toxoplasmosis | 20 | 0 | 0 | 20 |
| **TOTAL** | **378** | **14** | **1** | **393** |

[1] Scleroderma, Sjögren's, MCTD etc.
[2] Of the 10 medical condition specimens that were reactive on MONOLISA Anti-HBs EIA (excluding HIV-2 positive samples), 3 were reactive on the reference anti-HBs EIA, 5 were nonreactive, and 2 were QNS for additional testing
[3] Two specimens were reactive when tested with a reference anti-HBs EIA; 2 were QNS for additional testing
[4] Specimen was reactive when tested with a reference anti-HBs EIA

Of the 393 specimens from 20 unrelated medical conditions that were tested, 378/393 (96.2%) were nonreactive on the MONOLISA™ Anti-HBs EIA. Fourteen (14) specimens were reactive: 4 HIV-2 reactive specimens, 2 influenza vaccine specimens, 2 HCV positive specimens and 1 each of 8 other conditions (CMV, HAV, HIV-1, HTLV, Rubella, and SLE).

**Potentially Interfering Substances**

The MONOLISA™ Anti-HBs EIA was evaluated for interference according to CLSI Document EP7. None of the interferents at the levels tested below produced a change in clinical interpretation or a significant ≥ 10 % change of the assay.

Hemolyzed: 500 mg/dL of hemoglobin
Lipemic: 1000 mg/dL of triglycerides
Icteric: 20 mg/dL of bilirubin
Proteinemic: 15 g/dL of protein

The MONOLISA™ Anti-HBs assay did not detect a high-dose hook effect in patient samples with levels of antibodies to HBsAg as high as 217,000 mIU/mL. The MONOLISA™ Anti-HBs EIA is designed using a two-step format, where a high-dose hook effect is not normally observed.[21]

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## Reproducibility

A 7-member panel consisting of diluted patient specimens in various matrices (serum and EDTA) was tested in duplicate, once a day for 10 days, on 3 lots of the MONOLISA™ Anti-HBs EIA at 3 separate clinical trial sites.

The data from all 3 reagent lots were combined to obtain standard deviation (SD) and percent coefficient of variation (CV) for within run, between run, and total variance. The data were analyzed according to the principles described in CLSI EP5-A2 and ISO/TR 22971:2005. The data summary for this study is shown in Tables 12 and 13.

**Table 12**
**MONOLISA™ Anti-HBs EIA Reproducibility Results**
**by Panel Member Signal to Cutoff (S/CO)**

| Test Site | Panel Member | N | Mean S/CO | Within Run[1] | | Between Run[2] | | Total[3] | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | SD | CV (%) | SD | CV (%) | SD | CV (%) |
| Site #1 | 1 Pos Serum | 60 | 8.265 | 0.309 | 3.7 | 0.579 | 7.0 | 0.487 | 5.9 |
| | 2 ~12 mIU/mL (Serum) | 60 | 1.349 | 0.051 | 3.8 | 0.104 | 7.7 | 0.085 | 6.3 |
| | 3 ~8 mIU/mL (Serum) | 60 | 0.955 | 0.047 | 4.9 | 0.072 | 7.5 | 0.064 | 6.7 |
| | 4 Neg (Serum) | 60 | 0.288 | 0.012 | 4.3 | 0.073 | 25.3 | 0.054 | NA |
| | 5 ~12 mIU/mL (EDTA) | 60 | 1.435 | 0.032 | 2.2 | 0.090 | 6.3 | 0.078 | 5.5 |
| | 6 ~8 mIU/mL (EDTA) | 60 | 1.044 | 0.033 | 3.2 | 0.070 | 6.7 | 0.063 | 6.1 |
| | 7 Neg (EDTA) | 60 | 0.329 | 0.018 | 5.5 | 0.090 | 27.3 | 0.065 | NA |
| Site #2 | 1 Pos Serum | 60 | 8.008 | 0.259 | 3.2 | 0.984 | 12.3 | 0.696 | 8.7 |
| | 2 ~12 mIU/mL (Serum) | 60 | 1.289 | 0.078 | 6.1 | 0.136 | 10.5 | 0.109 | 8.5 |
| | 3 ~8 mIU/mL (Serum) | 60 | 0.950 | 0.089 | 9.3 | 0.225 | 23.6 | 0.165 | 17.4 |
| | 4 Neg (Serum) | 60 | 0.285 | 0.093 | 32.7 | 0.197 | 69.1 | 0.149 | NA |
| | 5 ~12 mIU/mL (EDTA) | 60 | 1.384 | 0.098 | 7.0 | 0.205 | 14.8 | 0.156 | 11.3 |
| | 6 ~8 mIU/mL (EDTA) | 60 | 0.971 | 0.037 | 3.8 | 0.085 | 8.7 | 0.068 | 7.0 |
| | 7 Neg (EDTA) | 60 | 0.292 | 0.058 | 19.9 | 0.149 | 50.9 | 0.117 | NA |
| Site #3 | 1 Pos Serum | 60 | 6.707 | 0.373 | 5.6 | 1.560 | 23.3 | 1.150 | 17.1 |
| | 2 ~12 mIU/mL (Serum) | 60 | 1.034 | 0.055 | 5.3 | 0.243 | 23.5 | 0.176 | 17.0 |
| | 3 ~8 mIU/mL (Serum) | 60 | 0.705 | 0.050 | 7.1 | 0.146 | 20.7 | 0.111 | 15.7 |
| | 4 Neg (Serum) | 60 | 0.249 | 0.061 | 24.3 | 0.130 | 52.0 | 0.098 | NA |
| | 5 ~12 mIU/mL (EDTA) | 60 | 1.124 | 0.093 | 8.2 | 0.299 | 26.6 | 0.218 | 19.4 |
| | 6 ~8 mIU/mL (EDTA) | 60 | 0.763 | 0.078 | 10.2 | 0.181 | 23.7 | 0.139 | 18.2 |
| | 7 Neg (EDTA) | 60 | 0.200 | 0.042 | 21.0 | 0.062 | 30.8 | 0.054 | NA |

NA = Not Applicable.
[1] Within Run: variability of the assay performance from replicate to replicate.
[2] Between Run: variability of the assay performance from run to run.
[3] Total variability of the assay performance includes within run, between run and between lot.

**Table 13**
**MONOLISA™ Anti-HBs EIA Reproducibility Results (Positive, Low Positive, and High Negative)**
**by Panel Member S/CO**

| Summary of Panel Members | N= | Mean S/CO | Between Lot | | Between Site[1] | | Total[2] | |
|---|---|---|---|---|---|---|---|---|
| | | | SD | CV | SD | CV | SD | CV |
| Positive Serum | 180 | 7.660 | 1.334 | 17.4 | 6.473 | 84.5 | 1.069 | 14.0 |
| ~12 mIU/mL (Serum) | 180 | 1.223 | 0.194 | 15.9 | 1.295 | 105.9 | 0.188 | 15.3 |
| ~8 mIU/mL (Serum) | 180 | 0.870 | 0.145 | 16.7 | 1.107 | 127.2 | 0.168 | 19.3 |
| ~12 mIU/mL (EDTA) | 180 | 1.314 | 0.207 | 15.7 | 1.292 | 98.3 | 0.211 | 16.0 |
| ~8 mIU/mL (EDTA) | 180 | 0.926 | 0.175 | 18.9 | 1.131 | 122.2 | 0.153 | 16.6 |

[1] Sites were nested within lots
[2] Total variability includes within run, between run, between lot, and between site.

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**Quantitative Precision**

A precision study was performed with the MONOLISA Anti-HBs EIA using quantitative panels prepared in serum and EDTA plasma. Each 7-member panel spanned the linear range of the assay. The 14 specimens were tested in triplicate for 20 days, and results are summarized in Table 14.

**Table 14**
**MONOLISA™ Anti-HBs EIA 20-Day Precision Results in mIU/mL**

| Panel Member | N | mIU/mL | Within-Run | | Between-Day | | Total | |
|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | CV% | SD | CV% | SD | CV% |
| Serum ~10 mIU/mL | 60 | 11.1 | 0.259 | 2.34 | 1.361 | 12.30 | 1.386 | 12.52 |
| Serum ~25 mIU/mL | 60 | 26.6 | 1.163 | 4.37 | 1.589 | 5.97 | 1.969 | 7.40 |
| Serum ~85 mIU/mL | 60 | 89.1 | 1.372 | 1.54 | 2.383 | 2.67 | 2.750 | 3.09 |
| Serum ~350 mIU/mL | 60 | 363.3 | 3.372 | 0.93 | 5.202 | 1.43 | 6.199 | 1.71 |
| Serum ~500 mIU/mL | 60 | 492.2 | 19.882 | 4.04 | 17.794 | 3.62 | 26.682 | 5.42 |
| Serum ~750 mIU/mL | 60 | 726.9 | 13.088 | 1.80 | 17.429 | 2.40 | 21.796 | 3.00 |
| Serum ~950 mIU/mL | 60 | 946.3 | 11.786 | 1.25 | 14.777 | 1.56 | 18.901 | 2.00 |
| EDTA Plasma ~10 mIU/mL | 60 | 12.4 | 0.596 | 4.80 | 1.263 | 10.17 | 1.396 | 11.24 |
| EDTA Plasma ~25 mIU/mL | 60 | 27.9 | 0.462 | 1.66 | 1.111 | 3.99 | 1.204 | 4.32 |
| EDTA Plasma ~85 mIU/mL | 60 | 92.5 | 1.055 | 1.14 | 2.281 | 2.47 | 2.513 | 2.72 |
| EDTA Plasma ~350 mIU/mL | 60 | 367.5 | 8.216 | 2.24 | 7.953 | 2.16 | 11.435 | 3.11 |
| EDTA Plasma ~500 mIU/mL | 60 | 496.0 | 11.173 | 2.25 | 19.743 | 3.98 | 22.685 | 4.57 |
| EDTA Plasma ~750 mIU/mL | 60 | 738.5 | 10.457 | 1.42 | 16.504 | 2.23 | 19.538 | 2.65 |
| EDTA Plasma ~950 mIU/mL | 60 | 940.1 | 20.127 | 2.14 | 13.425 | 1.43 | 24.193 | 2.57 |

## 15 – Quantitative Procedure MONOLISA™ Anti-HBs Calibrator Kit, Catalog # 25219

**INTENDED USE: The MONOLISA™ Anti-HBs Calibrator Kit is intended for quantitative determination of anti-HBs in human serum and EDTA or citrated plasma. The MONOLISA™ Anti-HBs Calibrator Kit is to be used only with the MONOLISA™ Anti-HBs EIA (Catalog # 25220).**

**Composition of the MONOLISA™ Anti-HBs Calibrator Kit**

| Component | | Contents |
|---|---|---|
| C2 | 0 mIU/mL Calibrator 1 vial (1.6 mL) | • 0 mIU/mL of anti-HBs antibodies of human origin<br>• PBS buffer with addition of bovine serum and blue dye.<br>• Preservative: ProClin® 950, 0.16% |
| C4 | 100 mIU/mL Calibrator 1 vial (1.6 mL) | • 100 mIU/mL of anti-HBs antibodies of human origin<br>• PBS buffer with addition of bovine serum and blue dye.<br>• Preservative: ProClin® 950, 0.16% |
| C5 | 400 mIU/mL Calibrator 1 vial (1.6 mL) | • 400 mIU/mL of anti-HBs antibodies of human origin<br>• PBS buffer with addition of bovine serum and blue dye.<br>• Preservative: ProClin® 950, 0.16% |
| C6 | 1000 mIU/mL Calibrator 1 vial (1.6 mL) | • 1000 mIU/mL of anti-HBs antibodies of human origin<br>• PBS buffer with addition of bovine serum and blue dye.<br>• Preservative: ProClin® 950, 0.16% |

The Calibrators are prepared from human anti-HBs specific immunoglobulins intended for therapeutic use, and are calibrated against WHO reference standard.

### WARNINGS FOR USERS

**For in vitro diagnostic use only.**

1. No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivates, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens.[14] Biosafety Level 2[15] or other appropriate biosafety practices[16,17] should be used for materials that contain or are suspected of containing infectious agents. The following human blood derivatives are found in Calibrator and/or EIA kit components:
   - 1.1 Human source material used in the preparation of the Negative Control (C0) and as a diluent for the Positive Control (C1) is nonreactive for detectable hepatitis B surface antigen (HBsAg), and antibodies to hepatitis B core antigen, hepatitis C virus (HCV), and human immunodeficiency viruses (HIV-1 and HIV-2).
   - 1.2 The human anti-HBs immunoglobulin used in the preparation of the Positive Control (C1) and Calibrator (C3) is a therapeutic grade material which has been inactivated.
   - 1.3 The human plasma derived viral antigen HBsAg subtypes ad and ay used in the preparation of the Microplate (R1) and Conjugate Concentrate (R4) are highly purified and heat treated.
2. The following is a list of potential chemical hazards contained in some kit components (See above table for Calibrator Kit components and section 4: REAGENTS for EIA kit components):
   - 2.1 ProClin® 300 (0.1% or 0.5%) or ProClin® 950 (0.16%) are biocidal preservatives that are irritating to eyes and skin, may be detrimental if enough is ingested, and may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
   - 2.2 0.005% Gentamicin Sulfate, a biocidal preservative, which is a known reproductive toxin, photosensitizer and sensitizer; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.
   - 2.3 The 1.0 N Sulfuric Acid ($H_2SO_4$) Stopping Solution is irritating to skin and severely irritating or corrosive to eyes, depending on the amount and length of exposure; greater exposures can cause eye damage, including permanent impairment of vision. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Keep away from strong bases, reducing agents and metals; do not pour water into this component. Waste from this material is considered hazardous acidic waste. However, if permitted by local, regional, and national regulations, it can be neutralized to pH 6-9 for non-hazardous disposal if operators are trained and equipped to do so.
3. Biological spills: Human source material spills should be treated as potentially infectious. Spills not containing acid should be immediately decontaminated, including the spill area, materials and any contaminated

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surfaces or equipment, with an appropriate chemical disinfectant that is effective for the potential biohazards relative to the samples involved (commonly a 1:10 dilution of bleach, 70-80% Ethanol or Isopropanol, an iodophor [such as 0.5% Wescodyne™ Plus], or a phenolic, etc.) and wiped dry.[18-20]

Spills containing acid should be appropriately absorbed (wiped up) or neutralized, wiped dry and then the area should be decontaminated with one of the chemical disinfectants; materials used to absorb the spill should be disposed of as biohazardous waste.

NOTE: DO NOT PLACE SOLUTIONS CONTAINING BLEACH INTO THE AUTOCLAVE.

4. Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory chemical or biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulations.

**PRECAUTIONS FOR USERS**

- Do not use calibrators beyond their stated expiration date.
- For the manual pipetting of the calibrators, use individual pipette tips to eliminate carryover of specimens.

**REAGENT STORAGE**

- Store the Anti-HBs Calibrator Kit at 2-8°C.

**THE QUANTITATIVE ASSAY PROCEDURE**

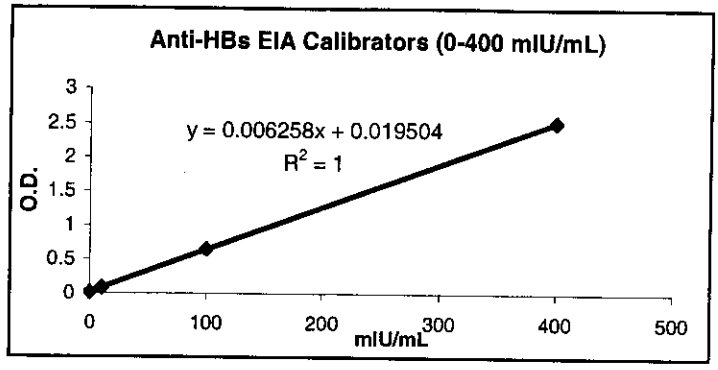
- The Calibrator Kit is used with the MONOLISA™ Anti-HBs EIA Kit (Catalog # 25220). Dilute each of the four Calibrators in the Calibrator Kit, as well as the EIA kit controls and 10 mIUmL Cutoff Calibrator, in the MONOLISA™ Anti-HBs EIA Specimen Diluent as described previously in Section 9, Anti-HBs EIA Procedure.
- The plate or partial plate is assayed as described in the EIA Procedure. Read the microwell plate using the 450 nm filter with 615 nm to 630 nm as the reference. (Blank on air.)
- For more concentrated specimens ($A_{450}$ values ≥ than that of the 400 mIU/mL calibrator) the microwell plate may also be read using the 405 nm filter with 615 nm to 630 nm as the reference.
- Patient serum or plasma specimens with concentrations greater than 1000 mIU/mL may be diluted in the Working Strength Wash Solution in the MONOLISA™ Anti-HBs EIA Kit, and re-assayed. For most specimens, a 1:10 or 1:100 predilution will bring the absorbance within the readable range at $A_{450}$ or $A_{405}$, although 1:1000 dilutions may be required for unusually high-titered specimens (>100,000 mIU/mL).

**QUANTITATIVE CALCULATIONS**

**$A_{450}$ Wavelength Reading (for specimens containing ≤ 400 mIU/mL)**

- The $A_{450}$ of three Calibrators from this Anti-HBs Calibrator Kit (0, 100, and 400 mIU/mL) along with the $A_{450}$ of the three separate 10 mIU/mL Cutoff Calibrator values from the MONOLISA™ Anti-HBs EIA kit are graphed versus their assigned concentrations, using linear regression[22]. **Please note that the $A_{450}$ of the 1000 mIU/mL calibrator cannot be used in this graph, as the absorbance value will be outside the range of the spectrophotometer.**
- $R^2$ and the equation of the line are determined. **$R^2$ should be ≥ 0.95**. Use the equation of the line to calculate the quantitative results of the specimens. Calculation of results is based on the formula [mIU/mL] = (O.D. – Intercept) / Slope. Scale should be set as appropriate to display all the absorbance values for calibrators up to 400 mIU/mL. See example calculations below. (For further assistance in calculating results using linear regression, contact the Bio-Rad Technical Support staff at 1-800-2BIORAD.)
- In addition to the quantitative results, specimens with calculated concentrations of **greater than 11 mIU/mL should be reported as reactive, those specimens with calculated concentrations of 9-11 mIU/mL should be reported as Borderline, and those with calculated concentrations of less than 9 mIU/mL should be reported as nonreactive**. Specimens with calculated mIU/mL results of less than 5 should be reported as <5 mIU/mL.

**Example Graph 1**

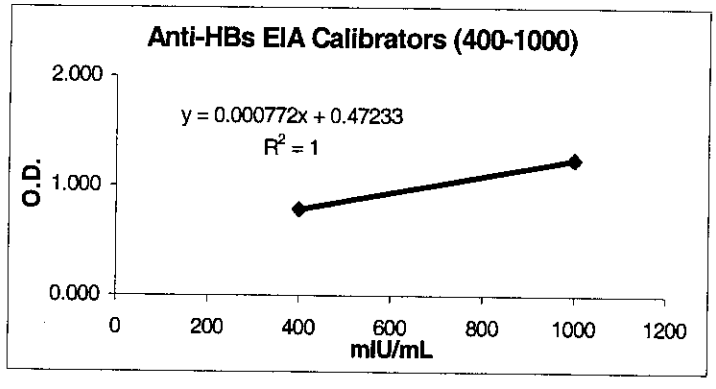



| Calibrators | |
|---|---|
| A450 | mIU/mL |
| 2.521 | 400 |
| 0.653 | 100 |
| 0.081 | 10 |
| 0.084 | 10 |
| 0.080 | 10 |
| 0.015 | 0 |

**$A_{405}$ Wavelength Reading (for specimens ≥ 400 mIU/mL and ≤ 1000 mIU/mL)**

- To determine the concentrations of specimens with higher concentrations of anti-HBs antibodies (≥ 400 mIU/mL to 1000 mIU/mL), the $A_{405}$ of two Calibrators from the Anti-HBs Calibrator Kit (1000 and 400, mIU/mL) can be graphed versus their assigned concentrations, using a linear regression. **The $A_{405}$ of the 1000 mIU/mL calibrator must be ≥ 1.3 X $A_{405}$ of the 400 mIU/mL calibrator**. The equation of the line is determined, and is used to calculate the concentrations of the specimens. The scale should be set as appropriate to display the absorbance values for calibrators 400 mIU/mL and 1000 mIU/mL.
- The $A_{405}$ curve is used to determine the concentrations of anti-HBs antibodies in serum or plasma specimens whose concentrations are ≥ 400 mIU/mL and ≤ 1000 mIU/mL. **This method is not recommended for quantitation of specimens containing < 400 mIU/mL. Use the A450 method described above.** Specimens with concentrations of anti-HBs antibodies greater than 1000 mIU/mL can be diluted in 1X Wash Solution in the MONOLISA™ Anti-HBs EIA Kit and re-assayed.

**Example Graph 2**




| Calibrators | |
|---|---|
| A405 | mIU/mL |
| 1.244 | 1000 |
| 0.781 | 400 |

**VALIDITY CRITERIA FOR THE QUANTITATIVE ASSAY PROCEDURE**

A run is valid if the following criteria for the qualitative assay are met:

- The absorbance value of the Positive Control must be greater than or equal to 0.600 AU (PC ≥ 0.600).
- The individual absorbance value of each 10 mIU/mL Cutoff Calibrator ($CAL_{10}i$) must be greater than or equal to 0.050 and less than or equal to 0.150 AU (0.050 ≤ $CAL_{10}i$ ≤ 0.150).
- The Cutoff Calibrators must be within the range of 0.65 to 1.35 times the Cutoff Calibrator mean absorbance value.
- The absorbance value of the Negative Control must be greater than 0.000 AU and less than or equal to 0.100 AU (0.000 < NC ≤ 0.100).
- The absorbance value of the Negative Control must be less than the mean of the absorbance values of the 10 mIU/mL Cutoff Calibrator, that is, less than the Cutoff Value (NC < $CAL_{10}\bar{x}$ ).

These additional criteria for the quantitative assay must also be met:

- The square of the correlation coefficient ($R^2$ ) of the $A_{450}$ calibrator line must be greater than or equal to 0.95.
- The absorbance values at $A_{450}$ and $A_{405}$ for the calibrators in each run must meet the criteria $Cal_{1000}$ > $Cal_{400}$ > $Cal_{100}$ > $Cal_{10}$ > $Cal_0$.
- When using the $A_{405}$ values for reading specimens ≥ 400 mIU/mL, the absorbance value of $Cal_{1000}$ must be ≥ 1.3 x the absorbance value of $Cal_{400}$ ($CAL_{1000}$ ≥ 1.3 x $CAL_{400}$).

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**Example:**

| Specimen | O.D. ($A_{450}$) | O.D. ($A_{405}$) | Results in mIU/mL | |
|---|---|---|---|---|
| Cal 0 | 0.015 | NA | -0.7 | Valid |
| Cal 10 | 0.080 | NA | 9.7 | Valid |
| Cal 10 | 0.084 | NA | 10.3 | Valid |
| Cal 10 | 0.081 | NA | 9.8 | Valid |
| Cal 100 | 0.653 | NA | 101.2 | Valid |
| Cal 400 | 2.521 | 0.794 | 399.7 | Valid |
| Cal 1000 | >3.000 (NA) | 1.244 | 1000 | Valid ($Cal_{1000}/Cal_{400}$ = 1.6) |
| Patient specimen #1 | 0.085 | NA | 10.5 (Borderline) | |
| Patient specimen #2 | 0.604 | NA | 93.4 (Reactive) | |
| Patient specimen #3 | >3.000 (NA) | 0.831 | 476.3 (Reactive) | |

Equation of $A_{450}$ line from Example Graph 1 (for specimens #1 and #2)

$y = 0.006258x + 0.019504$, where

$y$ = O.D. ($A_{450}$) and

$x$ = mIU/mL (unknown)

$x = (y - 0.019504) / 0.006258$

= 10.5 mIU/mL (specimen #1)

= 93.4 mIU/mL (specimen #2)

Equation of $A_{405}$ line from Example Graph 2 (for specimen #3)

$y = 0.000772x + 0.472333$, where

$y$ = O.D. ($A_{405}$) and

$x$ = mIU/mL (unknown)

$x = (y - 0.472333) / 0.000772$

= 476.3 mIU/mL (specimen #3)

**Interpretation**

**Borderline:** Specimens with levels of quantitative anti-HBs antibody between 9-11mIU/mL should be interpreted as borderline, as the specific immune status for those patients can't be determined without other clinical information or subsequent testing. Borderline results may indicate a low level of antibody that has clinical significance. Specimens that are borderline can be retested or the subject can be re-collected in 2-3 weeks for additional testing. In conjunction with these results, the immune status of subjects should be evaluated based on their clinical status, related risk factors, and other diagnostic test results.

**Reactive:** Specimens with absorbance values greater than the borderline zone (>11 mIU/mL) are considered reactive, and the patient is considered to be immune to infection with HBV. It has not been determined what the clinical significance is for values greater than 11 mIU/mL, other than the individual is considered to be immune to HBV infection.

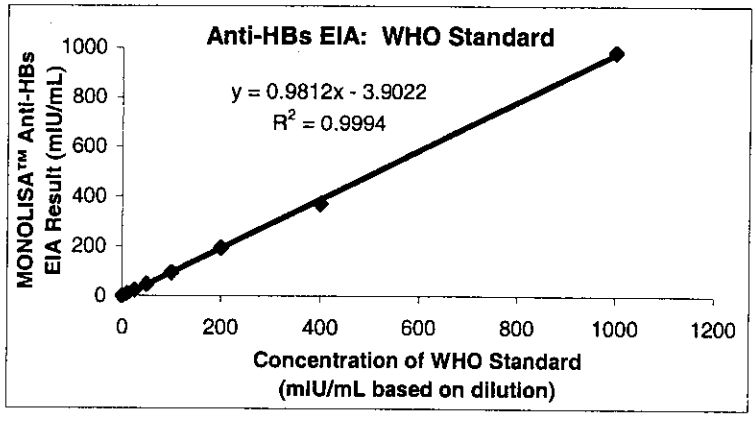
**Nonreactive:** Specimens with quantitative antibody values less than 9 mIU/mL are considered nonreactive, and the patient is considered to be not immune to infection with HBV. The quantitative antibody value of a specimen must be compared to the borderline zone determined for the plate on which it is assayed.

Specimens with absorbance values that are less than 0.000 must be repeated. Those with values greater than the upper linearity limits of the reader should be reported as reactive.

**Quantitative Results for WHO Standard**

A series of dilutions of the WHO Standard (WHO First International Reference Preparation for Antibody to HBsAg,1977) were tested in duplicate on the MONOLISA™ Anti-HBs EIA. The dilutions tested were 1000 mIU/mL, 400 mIU/mL, 200 mIU/mL, 100 mIU/mL, 50 mIU/mL, 10 mIU/mL, and 0 mIU/mL. The mIU/mL values for each of the dilutions was calculated from the calibration curve, as well as the anti-HBs concentration at the assay cutoff. Note: To determine the concentration of specimens with >400 mIU/mL of anti-HBs antibodies, the assay was read at $A_{405}$ nm.

**Chart 3**
**Calculated Results of WHO Standard Using Calibration Curve**



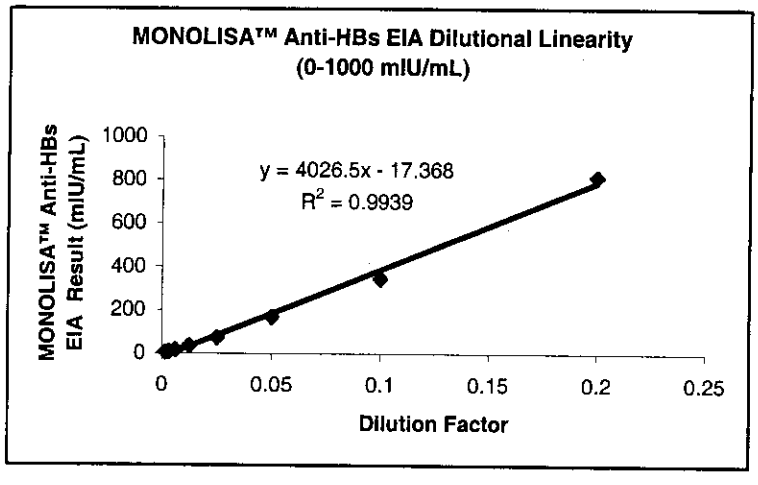

| | Anti-HBs |
|---|---|
| WHO Std | Calculated |
| mIU/mL | mIU/mL |
| 1000 | 984.4 |
| 400 | 369.7 |
| 200 | 193.7 |
| 100 | 93.6 |
| 50 | 47.0 |
| 25 | 23.2 |
| 10 | 9.1 |
| 0 | 0.0 |

assay cutoff = 9.9 mIl/mL (WHO Standard)

### Linearity

The linearity range (0-1000 mIU/mL) of MONOLISA™ Anti-HBs EIA was assessed by diluting a high-titer patient specimen pool into negative plasma and testing each dilution in duplicate.

**Chart 4**
**Dilutional Linearity of MONOLISA™ Anti-HBs EIA**




| Dilution | mIU/mL |
|---|---|
| 1:5 | 813.3 * |
| 1:10 | 343.1 |
| 1:20 | 168.8 |
| 1:40 | 73.1 |
| 1:80 | 35.8 |
| 1:160 | 16.2 |
| 1:320 | 9.7 |
| 1:640 | 5.4 |

* calculated from A405 curve

### Limit of Detection

The limit of detection was determined for the MONOLISA™ Anti-HBs EIA by testing 10 replicates of the WHO Standard diluted to 10 mIU/mL and 5 mIU/mL, and Specimen Diluent (0 mIU/mL). In these studies, the lowest amount of anti-HBs in a specimen that could be detected with a 95% probability was calculated to be 4.14 mIU/mL of anti-HBs.

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